Appendix 1

Table I: Relative Selectivity of Y639F and W.T. Polymerase for rNTPs vs. dNTPs

	rATP/dATP	rUTP/dTTP	rCTP/dCTP	rGTP/dGTP
Y639F	8.5-10 (Mg)*	1.7-1.9	2.4-2.5	.93-1.6
	.9-1.0 (Mn)*	.48-.76	.55-.80	.98-.99
W.T.	72-83 (Mg)*	22-25	110-150	51-67
	6-14 (Mn)*	2.3-2.8	4.3-5.1	4.4-6.3

Reactions were carried out with all 4 rNTPs (.5 mM) in great excess over radioactive rNTPs or dNTPs. Relative selectivity was determined from the relative percentages of radioactive rNTP vs. dNTP incorporated into RNA. Maximal incorporation was less than ~30% of total input radioactivity with all data points used so as to limit effects due to changing NTP concentrations during the experimental time course. The numbers shown give the range for 2 experiments.

*For each rNTP/dNTP and polymerase the upper numbers are those obtained in Mg^{++} buffer, the lower numbers are from Mn^{++} buffer. Polymerases at 10^{-8} M. Template was supercoiled pT75 at 10^{-7} M.

Table II: Relative Activity of Y639F and W.T. Polymerase with Different rNTP/dNTP Mixes

	W. T. (Mg^{++})	Y639F (Mg^{++})	W.T. (Mn^{++})	Y639F (Mn^{++})
4 rNTPs	200	200	21-23	9
3 rNTPs, dTTP	11-13	90-96	7-8	4
3 rNTPs, dUTP	9-11	82-91	10-11	7-8
3 rNTPs, dATP	1-2	73	1-2	2-3
3 rNTPs, dCTP	4-9	86	15	7-11
3 rNTPs, dGTP, rGMP	< .5	95-109	1-3	1.4-2.5
3 rNTPs, dGTP	< .5	43-63	.5-2	3
2 rNTPs, dCTP, dUTP	2-6	27-29	3	4-7
2 rNTPs, dCTP, dTTP	2	30	5-7	5
2 rNTPs, dCTP, dATP	< .5	20-29	.5-.9	4-7
2 rNTPs, dTTP, dGTP, rGMP	< .5	13-15	< .5	3-6
2 rNTPs, dATP, dTTP	< .5	11-14	< .5	3-4
2 rNTPs, dCTP, dGTP, rGMP	< .5	11-14	< .5	2-5
2 rNTPs, dATP, dGTP, rGMP	< .5	5-6	< .5	1-1.5
1 rNTP, dCTP, dATP, dTTP	< .5	12-14	< .5	.7-2
1 rNTP, dCTP, dATP, dUTP	< .5	10-11	< .5	2-3
1 rNTP, dTTP, dCTP, dGTP*	< .5	10-13	< .5	2-4
1 rNTP, dTTP, dATP, dGTP*	< .5	11	< .5	1.5-2
1 rNTP, dCTP, dATP, dGTP*	< .5	3-5	< .5	1-2
4 dNTPs, rGMP	< .5	< .5	< .5	.5

*These reactions also contain rGMP. Numbers give ranges from 2 experiments. Template was supercoiled pT75 (10^{-7} M), polymerases at 10^{-8} M (in Mg^{++} buffer) or 10^{-7} M (in Mn^{++} buffer). rNTPs, rGMP, dTTP were at .5 mM; dATP, dGTP were at 1 mM; dUTP was at 2.5 mM, dCTP was at 5 mM. From top to bottom the labeling NTPs were: α-P^{32} rGTP, α-P^{32} rCTP, α-P^{32} rCTP, α-P^{32} dATP, α-P^{32} dCTP, α-P^{32} dGTP, α-P^{32} dGTP, α-P^{32} dCTP, α-P^{32} dTTP, α-P^{32} dCTP, α-P^{32} dTTP, α-P^{32} dTTP, α-P^{32} dCTP, α-P^{32} dATP, α-P^{32} dTTP, α-P^{32} dCTP, α-P^{32} dTTP, α-P^{32} dTTP, α-P^{32} rCTP, α-P^{32} dCTP.

Table III: Relative activity on poly(dI)•poly(dC)

	W.T.	Y639F	G640A	Y639A	Y639S
rGTP	1000	1000	240 (200-270)	145 (142-151)	48 (47-50)
dGTP	7.4 (5.4-12.5)	964 (684-1257)	<5	5.3 (4.8-5.5)	.6
dGTP + rGMP	25 (20-27)	1070 (816-1457)	<5	25 (17-30)	4.4

Numbers give mean and range from 3 experiments. Templates were at .2 mg/ml, polymerases at 10^{-8} M. Labeling NTPs were α-P32 rGTP, α-P32 dGTP, α-P32 rATP, α-P32 dATP, as appropriate. rNTPs or rNMPs at .5 mM; dNTPs at 1 mM.

Table IV: W.T. and Y639F activity on an RNA (poly(rC)) template

	.5 mM GTP	1 mM dGTP	1 mM dGTP+.5 mM GMP
w.t.	1000	<.5	<.5
Y639F	505 (358-733)	62 (48-80)	116 (90-148)

Numbers give mean and range from 3 experiments. Template was at .2 mg/ml, polymerases at 10^{-6} M. Labeling NTPs were α-P32 rGTP, α-P32 dGTP.